Mycorrhizal infection and high soil phosphorus improve vegetative growth and the female and male functions in tomato

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Summary

• To further characterize the effects of mycorrhizal infection and soil phosphorus (P) availability on plant fitness, this study examined their effects on the female and male functions, as well as vegetative growth of tomato (*Lycopersicon esculentum*).

• Two cultivars of tomato were grown in a glasshouse under three treatment combinations: nonmycorrhizal, low P (NMPO); nonmycorrhizal, high P (NMP3); and mycorrhizal, low P (MPO).

• Mycorrhizal infection and high soil P conditions improved several vegetative (leaf area, days until first flower and leaf P concentration) and reproductive traits (total flower production, fruit mass, seed number and pollen production per plant, and mean pollen production per flower). In general, mycorrhizal and P responses were greater for reproductive traits than vegetative traits. In one cultivar, these responses were greater for the male function than the female function.

• Thus, mycorrhizal infection and high soil P conditions enhanced fitness through both the female and male functions. Similar trends were usually observed in the NMP3 and MPO treatments, suggesting that mycorrhizal effects were largely the result of improved P acquisition.

Key words: *Lycopersicon esculentum*, fitness, fruit set, mycorrhiza, pollen, resource allocation, soil fertility, vegetative growth.

Introduction

Despite the large body of research on arbuscular mycorrhizal (AM) effects on vegetative growth, relatively little attention has been given to its effects on plant reproduction, especially the male function. Because most plants are hermaphrodites (Charnov, 1982), contributing genes to the next generation through both the male and female functions, estimates of reproductive success through the male function are just as important as estimates of success through the female function. Several studies have demonstrated that the male and female functions do not always respond in a similar manner (Horovitz & Harding, 1972; Bertin, 1982). Thus, it cannot be assumed that reproduction will show the same level of response to mycorrhizal infection and high soil P conditions as vegetative growth, or that the male and female functions of reproduction will respond in similarly.

Because reproductive output is often highly correlated with plant size and nutrient status (Harper & White, 1974; Solbrig, 1981), it is not surprising that mycorrhizal plants usually produce more seeds and fruit than nonmycorrhizal plants (Jensen, 1982, 1983; Schenck & Smith, 1982; Dodd *et al.*, 1983; Koide *et al.*, 1988; Bryla & Koide, 1990a; Carey *et al.*, 1992; Stanley *et al.*, 1993; Vejsadova *et al.*, 1993; Bethlenfalvay *et al.*, 1994, 1997; Lu & Koide, 1994; Subramanian & Charest, 1997). Furthermore, mycorrhizal infection can improve seed quality (seed size and P status) in addition to seed quantity (Koide *et al.*, 1988; Bryla & Koide, 1990a; Lu & Koide, 1991; Koide & Lu, 1992; Lu, 1993; Stanley *et al.*, 1993).

Beyond seed and fruit production, relatively little attention has been given to the effects of mycorrhizal infection on other aspects of plant reproduction. Mycorrhizal infection reduced the time until first flower in tomato (*Lycopersicon esculentum*) and *Abutilon theophrasti* (Bryla & Koide, 1990a; Lu & Koide, 1994). In addition, mycorrhizal infection increased flower bud production in pepper (Dodd *et al.*, 1983) and flower production in soybean (*Glycine max*), tomato, and *A. theophrasti* (Schenk & Smith 1982; Bryla & Koide, 1990a; Lewis & Koide, 1990; Lu & Koide, 1994). Mycorrhizal infection also increased the percentage of flowers producing fruits in tomato and *A. theophrasti* (Bryla & Koide, 1990a; Lu & Koide, 1994).

Since both seeds and pollen depend on the sporophyte for provisioning of nutrients, it is reasonable to expect that mycorrhizal infection and high soil P conditions could improve the male function of reproduction as well as the female function. Past studies have found a positive relationship between environmental factors that affect resource availability (e.g. soil fertility and leaf herbivory) and total pollen production, pollen production per flower, and pollen grain size (Vasek *et al.*, 1987; Devlin, 1989; Young & Stanton, 1990; Stephenson *et al.*, 1992; Lau & Stephenson, 1993, 1994; Stephenson *et al.*, 1994; Quesada *et al.*, 1995; Johannsson & Stephenson, 1998; Stephenson *et al.*, 1998). Furthermore, environmental factors can affect the chemical composition of pollen (Stanley & Linskens, 1974; Baker & Baker, 1979; van Herpen & Linskens, 1981; Lau & Stephenson, 1994; Stephenson *et al.*, 1998). For example, high soil P conditions in the field had an overall positive effect on the male function (staminate flower production, pollen production per flower, pollen grain size, and pollen P concentration) in *Cucurbita pepo* (Lau & Stephenson, 1994). In a preliminary glasshouse study with *C. pepo*, mycorrhizal plants produced marginally more pollen grains per staminate flower and significantly larger pollen grains than nonmycorrhizal plants, with these differences increasing over an 8-wk period (Lau *et al.*, 1995). In another study with *C. pepo* grown in the field, pollen from mycorrhizal plants showed faster *in vitro* pollen tube growth rates than pollen from nonmycorrhizal plants (Stephenson *et al.*, 1998). Recent studies with other species have shown further beneficial effects of mycorrhizal infection on pollen production (Pendleton, 2000; Philip *et al.*, 2001; Poulton *et al.*, 2001b) and pollen performance (Poulton *et al.*, 2001a,b).

In the study reported here, two tomato cultivars were grown in a glasshouse under three treatment combinations: nonmycorrhizal, low P (NMPO); nonmycorrhizal, high P (NMP3); and mycorrhizal, low P (MPO). The effects of mycorrhizal infection and soil P availability were determined for both vegetative (leaf area, days until first flower – a measure of pre-reproductive growth rate – leaf P concentration, and final leaf biomass) and reproductive traits (total flower production per plant), including separate measures of the female (total fruit mass per plant, fruit abortion, fruit P concentration, mean seed number per fruit and total seed number per plant), and male function (mean pollen production per flower, total pollen production per plant and pollen grain size). Although individual responses to mycorrhizal infection and soil P availability are common in the literature, few studies compare vegetative, female, and male responses within a single study, as described here.

Materials and Methods

In a glasshouse study, two cultivars of tomato (*Lycopersicon esculentum* Mill.), 'VF36' and 'VFNT Cherry', were used to examine the effects of mycorrhizal infection and soil P availability on vegetative growth and the female and male functions of reproduction. Both cultivars were obtained from the Tomato Genetics Research Center at the University of California, Davis, CA, USA. 'VF36' produces two to four flowers per inflorescence and large fruit (mean fruit mass = 6.2 g); 'VFNT Cherry' produces six to eight flowers per inflorescence and small fruit (mean fruit mass = 0.7 g). Both cultivars are self-compatible and show indeterminate growth and reproduction. These cultivars were selected because both have mutations for stem color that can be used to determine paternity in pollen mixture studies (Poulton *et al.*, 2001a).

Soil was collected from a low P (14 ppm Olsen extractable P) field at the Pennsylvania State University Agricultural Experiment Station at Rock Springs, PA, USA. Indigenous mycorrhizal fungi were destroyed by autoclaving the air-dried soil at 105°C for 90 min Then the soil was stored for 2 wk to avoid the potentially phytotoxic effects of autoclaving (Rovira & Bowen, 1966). In order to improve drainage, the field soil was mixed in a 1 : 3 ratio with sterile medium-grade sand.

On 4 June 1997, 'VF36' and 'VFNT Cherry' tomato seeds were planted in trays containing 50% PGX growing mix (Premier, Riviere-du-Loup, Quebec, Canada), 40% Perlite, and 10% whole-soil inoculum (approx. 75 spores of *Glomus etunicatum* Becker and Gerd. per ml of inoculum) for mycorrhizal seedlings, or 10% autoclaved low P field soil (same soil used to produce inoculum) for nonmycorrhizal seedlings. On 23 June, two seedlings were transplanted into each 'Azalea' pot (3800 cm^3; Kord, Bramalea, Ontario, Canada), containing the soil–sand mixture. Mycorrhizal seedlings were inoculated again with 60 ml whole soil inoculum directly around their roots; nonmycorrhizal seedlings received 60 ml autoclaved low P field soil directly around their roots with 5 ml spore washings to obtain comparable nonmycorrhizal microbial inputs (Koide & Li, 1989). Pots were arranged in a randomized block design across three benches. (Two benches had three blocks each; one bench had two blocks.) Soil P treatment was established by watering plants once per week with 500 ml of one-third strength Hoagland's nutrient solution (Machlis & Torrey, 1956) without P for 'low P' plants and with 1 mM KH_2PO_4 for 'high P' plants. A preliminary P

response study had determined the concentration of KH_2PO_4 required for nonmycorrhizal plants to have similar vegetative growth to mycorrhizal plants (Poulton, 2000). This resulted in three treatment combinations: nonmycorrhizal, low P (NMPO); nonmycorrhizal, high P (NMP3); and mycorrhizal, low P (MPO). A drip irrigation system supplied plants with additional water as needed. At 9 wk after transplanting, 400 ml water was supplied to each plant daily (except on the days when Hoagland's solution was provided). Thus, there were 48 pots, with eight replicates per cultivar–treatment combination. This study was performed at the same time as the study in Poulton *et al.* (2001b); some plants were used in both studies.

One randomly selected plant from each pot was removed at 3 wk after transplanting (leaving one plant per pot). At 5 wk after transplanting, a root sample was taken midway between the stem and pot edge in each pot with a no. 15 cork bore. The hole was filled with the autoclaved soil–sand mixture. Roots were rinsed out of the soil samples and initially stored in formaldehyde–acetic acid–ethanol (FAA) solution. Then the roots were cleared and stained with trypan blue to determine level of mycorrhizal infection (NMPO, NMP3 ≈ 0%; MPO = 68%) using the modified gridline intersect technique in Koide & Mooney (1987).

The vegetative response to the three treatments was measured as leaf area, days until first flower, leaf P concentration, and final leaf biomass. Starting at 3 wk after transplanting, leaf area measurements were recorded once per week for 3 wk, as described in Bryla & Koide (1990b) and Poulton *et al.* (1998). The first plants began to flower 4 wk after transplanting. For each plant, the date on which its first flower opened was recorded. At 5 wk after transplanting, one fully expanded leaf was collected from each plant for P analysis (the youngest leaf greater than 12 cm long for 'VF36' and 9 cm in long for 'VFNT Cherry'). Leaves were dried at 70°C to constant weight and then ground to obtain subsamples. Weighed subsamples were digested in a H_2SO_4–H_2O_2 mixture using a Technicon (Tarrytown, NY, USA) block digester (Bryla & Koide, 1990a,b). Then the digested subsamples were analysed for P using the molybdo-phosphate method (Watanabe & Olsen, 1965). Ground leaf tissue from sunflowers was used as reference material to check digestion and analytical procedures with each set of 40 samples. At 18 wk after transplanting, all leaves were harvested from each plant and dried at 70°C to constant weight. Then they were weighed to determine final leaf biomass.

The overall reproductive response to the three treatments was measured as total flower production per plant. In addition, separate measures of the female (total fruit mass per plant, fruit abortion, fruit P concentration, mean seed number per fruit and total seed number per plant) and male function (mean pollen production per flower, total pollen production per plant and pollen grain size) were evaluated. Total flower production per plant was determined by counting the number of flowers produced per month for the remaining 3 months of the study. All open flowers were vibrated twice per week with a modified electric toothbrush to ensure fruit production via self-pollination. Vibrating a flower at a high frequency causes pollen to be released from the anther cone, saturating the stigmatic surface within the cone. Starting at 6 wk after transplanting, pollen was collected weekly to determine mean pollen production per flower. On each plant, all open flowers were vibrated, and pollen for that plant was collected in a single gelatin caplet. Pollen samples were dried at 40°C and stored in 20-ml vials for future analysis. Individual pollen samples were then weighed on a scale with precision to 0.001 mg. Mean pollen production per flower was calculated by dividing mass of the pollen sample by the number of flowers vibrated on that plant. Pollen counts performed on microscope slides showed a strong correlation ($R^2 = 0.97$, $n = 36$) with mass (Poulton, 2000). Total pollen production per plant was calculated as number of flowers × mean pollen production per flower. Pollen samples were collected monthly to measure pollen grain size. One pollen load was transferred on the flat end of a wire to a microscope slide and was stained with acetocarmine (Kearns & Inouye, 1993). On each slide, the diameters of the first 20 pollen grains encountered on a grid were measured with a micrometer. As fruit ripened, they were harvested and dried at 70°C to constant weight. Dried fruit were weighed to determine total fruit mass per plant. Fruit abortion was calculated as ((number of flowers – number of mature fruit)/number of flowers) × 100. The number of seeds per fruit was recorded from a representative sample of the fruit (30% of the fruit produced by each cultivar–treatment combination). Total seed production per plant was calculated as number of fruit × mean seed number per fruit. Fruit P concentration was determined as previously described for leaf tissue.

For most vegetative and reproductive traits, fixed effects analyses of variance (general linear model; Minitab, 1997) were performed with two factors and their interaction: cultivar (two levels) and treatment (three levels). Time (three levels) was also included in the analysis of variance for leaf area. Least square means were calculated whenever sample size varied among cultivar–treatment combinations. Bonferroni pairwise comparisons were used to detect significant differences among the treatments within a cultivar. In addition, multivariate analyses of variance (General MANOVA; Minitab, 1997) were performed for vegetative traits, measures of the female function and measures of the male function, in order to detect overall significant treatment effects. Total flower production per plant was included in both MANOVAs for reproductive traits. Mycorrhizal response was calculated as ((MPO – NMPO mean)/NMPO mean) × 100 (Bryla & Koide, 1990b). Similarly, P response was calculated as ((NMP3 – NMPO mean)/NMPO mean) × 100. Leaf area (at 5 wk), total flower production per plant, total seed production per plant and total pollen production per plant were used to

Table 1 Analysis of variance of leaf area (measured at weeks 3, 4 and 5) in 'VF36' and 'VFNT Cherry'

Source	df	*F*	*P*
Time	2	184.71	0.001
Cultivar	1	1763.77	0.001
Treatment	2	176.83	0.001
Time × cultivar	2	50.99	0.001
Time × treatment	4	5.87	0.001
Cultivar × treatment	2	19.21	0.001
Time × cultivar × treatment	4	0.48	0.749

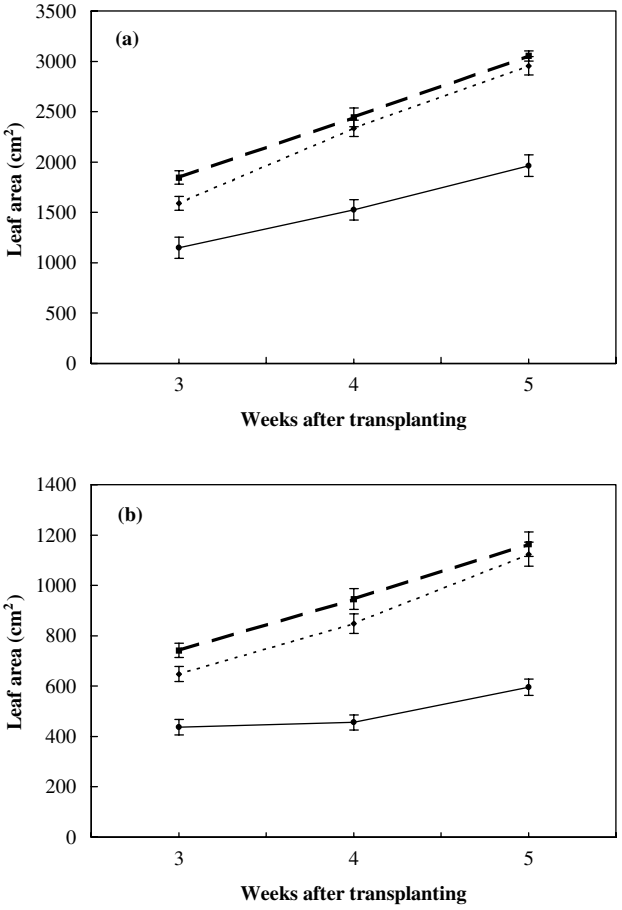

Fig. 1 Leaf area (measured at weeks 3, 4, and 5) in (a) 'VF36' and (b) 'VFNT Cherry'. Means (± SE) are given. Solid line, NMPO (nonmycorrhizal, low P); large dashes, NMP3 (nonmycorrhizal, high P); small dashes, MPO (mycorrhizal, low P).

evaluate plant response to mycorrhizal infection and high soil P conditions.

Results

As expected, cultivar had a significant effect on most vegetative and reproductive traits throughout this study. In both cultivars, NMP3 and MPO plants had greater leaf area than NMPO plants, with these differences among treatments increasing over time (Table 1, Fig. 1). Treatment also had a significant effect on days until first flower (Table 2). In the cv. VF36, NMP3 and MPO plants began to flower significantly faster than NMPO plants. In the cv. VFNT Cherry, NMP3 plants began to flower significantly faster than NMPO plants (MPO plants were intermediate). Treatment also had a significant effect on leaf P concentration and final leaf biomass (Table 2). In both cultivars, leaves from NMP3 plants had a significantly higher P concentration than leaves from NMPO plants (MPO plants were intermediate). In addition, NMP3 plants had greater final leaf biomass than NMPO and MPO plants. In both cultivars, the relationship between leaf area and leaf P concentration at week 5 varied among the treatments, although there was always a positive correlation ('VF36' $R^2 = 0.55$; 'VFNT Cherry' $R^2 = 0.66$; Fig. 2). In the multivariate analysis of variance, there was an overall significant treatment effect for vegetative traits, especially in 'VFNT Cherry' (MANOVA, $F = 18.88$, $P = 0.001$).

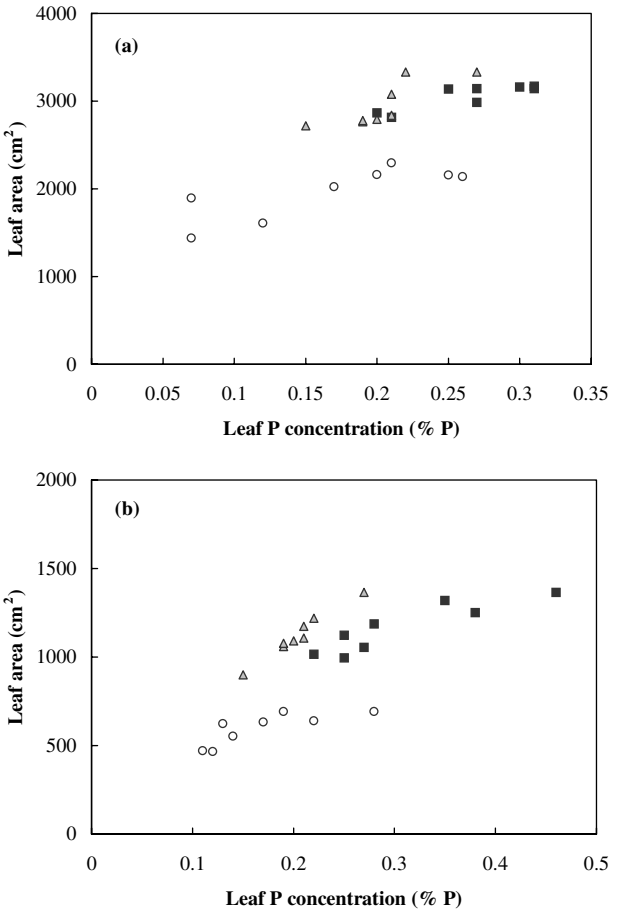

Fig. 2 Relationship between leaf area (cm^2) and leaf P concentration at week 5 in (a) 'VF36' and (b) 'VFNT Cherry'. Circles, NMPO (nonmycorrhizal, low P); squares, NMP3 (nonmycorrhizal, high P); triangle, MPO (mycorrhizal, low P).

Table 2 Vegetative traits in 'VF36' and 'VFNT Cherry'. Days until first flower, leaf P concentration (at week 5), and final leaf biomass (g) are given as means and SE (in parentheses)

Cultivar	Treatment	Days until first flower	Leaf P concentration	Final leaf biomass
VF36	NMPO	47.1 (2.6)a	0.17 (0.03)a	4.9 (0.8)a
	NMP3	37.4 (0.5)b	0.27 (0.02)b	8.2 (0.7)b
	MPO	40.9 (0.8)b	0.21 (0.01)ab	5.5 (0.4)a
VFNT Cherry	NMPO	37.0 (2.9)a	0.17 (0.02)a	3.0 (0.3)a
	NMP3	29.0 (0.6)b	0.31 (0.03)b	3.7 (0.3)b
	MPO	34.9 (1.1)ab	0.25 (0.03)ab	2.5 (0.2)a
ANOVA *P*-values	Cultivar	0.001	0.108	0.001
	Treatment	0.001	0.001	0.001
	Cultivar × treatment	0.489	0.541	0.052

Different letters within a cultivar indicate a significant difference at $P = 0.05$. NMPO, nonmycorrhizal, low P; NMP3, nonmycorrhizal, high P; MPO, mycorrhizal, low P.

Table 3 Measures of the female function of reproduction in the 'VF36' and 'VFNT Cherry' cultivars. Total flower production per plant, total fruit mass per plant (g), fruit abortion and fruit P concentration are given as means and SE (in parentheses); mean seed number per fruit and total seed number per plant are given as least square means and SE (in parentheses)

Cultivar	Treatment	Total flower production per plant	Total fruit mass per plant	Fruit abortion
VF36	NMPO	7.9 (1.5)a	9.1 (1.4)a	75.9 (5.8)a
	NMP3	12.8 (1.5)b	18.6 (1.3)b	71.1 (3.3)a
	MPO	12.5 (1.7)b	13.6 (1.3)ab	81.0 (4.5)a
VFNT Cherry	NMPO	29.0 (3.3)a	5.2 (1.3)a	68.9 (6.8)a
	NMP3	61.3 (3.8)c	16.8 (1.2)b	59.8 (1.9)a
	MPO	42.3 (3.7)b	13.9 (0.5)b	53.3 (4.8)a
ANOVA *P*-values	Cultivar	0.001	0.078	0.001
	Treatment	0.001	0.001	0.320
	Cultivar × treatment	0.001	0.249	0.084
		Fruit P concentration	Mean seed number per fruit	Total seed number per plant
VF36	NMPO	0.22 (0.02)a	89.8 (18.0)a	130.2 (45.0)a
	NMP3	0.38 (0.02)b	75.8 (11.6)a	273.1 (35.6)b
	MPO	0.25 (0.03)a	89.4 (15.2)a	195.0 (45.0)ab
VFNT Cherry	NMPO	0.17 (0.02)a	38.4 (4.8)a	392.6 (110.0)a
	NMP3	0.29 (0.01)b	54.3 (2.8)b	1300.9 (102.9)c
	MPO	0.22 (0.02)a	48.0 (2.9)ab	879.4 (102.9)b
ANOVA *P*-values	Cultivar	0.001	0.001	0.001
	Treatment	0.001	0.805	0.001
	Cultivar × treatment	0.212	0.091	0.001

Different letters within a cultivar indicate a significant difference at $P = 0.05$. NMPO, nonmycorrhizal, low P; NMP3, nonmycorrhizal, high P; MPO, mycorrhizal, low P.

Treatment had a significant effect on total flower production per plant, especially in 'VFNT Cherry' (Table 3). In the 'VF36', NMP3 and MPO plants produced significantly more flowers than NMPO plants. In 'VFNT Cherry', NMP3 plants produced significantly more flowers than MPO plants, which in turn produced significantly more flowers than NMPO plants (i.e. NMP3 > MPO > NMPO). Treatment also had a significant effect on total fruit mass per plant (Table 3). In 'VF36', NMP3 plants had significantly greater total fruit mass than NMPO plants (MPO plants were intermediate). In 'VFNT Cherry', NMP3 and MPO plants had significantly greater total fruit mass than NMPO plants. Treatment did not have a significant effect on fruit abortion (Table 3). However, there was a significant treatment effect on fruit P concentration (Table 3). In both cultivars, fruit from NMP3 plants had a significantly higher P concentration than fruit from NMPO and MPO plants. Treatment also had a significant effect on total seed number per plant, especially in 'VFNT Cherry' (Table 3). In 'VF36', NMP3 plants produced significantly more seeds than NMPO plants (MPO plants were intermediate). In 'VFNT Cherry', NMP3 plants produced significantly more seeds than MPO plants, which in

Table 4 Measures of the male function of reproduction in the VF36 and VFNT Cherry cultivars. Mean pollen production per flower (mg), total pollen production per plant (mg), and pollen grain size (microns) are given as least square means and SE (in parentheses)

Cultivar	Treatment	Mean pollen production per flower	Total pollen production per plant	Pollen grain size
VF36	NMPO	0.132 (0.063)a	1.26 (0.81)a	28.3 (0.4)a
	NMP3	0.329 (0.063)b	4.75 (0.89)b	28.3 (0.4)a
	MPO	0.284 (0.055)b	3.63 (0.70)b	27.7 (0.4)a
VFNT Cherry	NMPO	0.025 (0.009)a	0.86 (0.43)a	25.4 (0.3)a
	NMP3	0.039 (0.006)ab	2.41 (0.34)b	25.7 (0.2)a
	MPO	0.054 (0.007)b	2.26 (0.36)b	25.8 (0.2)a
ANOVA *P*-values	Cultivar	0.001	0.010	0.001
	Treatment	0.022	0.001	0.675
	Cultivar × treatment	0.071	0.328	0.283

Different letters within a cultivar indicate a significant difference at $P = 0.05$. NMPO, nonmycorrhizal, low P; NMP3, nonmycorrhizal, high P; MPO, mycorrhizal, low P.

Table 5 Mycorrhizal and P responses (%) of selected vegetative and reproductive traits in 'VF36' and 'VFNT Cherry'. Least square means and (SE) of responses and *P*-values from analyses of variance are given

Trait	'VF36' Mycorrhizal response	*P*	P response	*P*	'VFNT Cherry' Mycorrhizal response	*P*	P response	*P*
Leaf area (at 5 weeks)	50.5 (4.6)	0.001	55.4 (2.6)	0.001	88.6 (8.0)	0.001	95.3 (8.3)	0.001
Total flower production per plant	58.2 (20.9)	0.013	61.4 (18.9)	0.007	45.7 (12.6)	0.004	111.2 (13.2)	0.001
Total seed production per plant	49.8 (32.9)	0.100	109.8 (31.7)	0.005	123.8 (13.9)	0.001	231.0 (34.0)	0.001
Total pollen production per plant	188.1 (49.7)	0.003	277.0 (107.0)	0.031	162.5 (60.3)	0.018	180.4 (26.5)	0.001

turn produced significantly more seeds than NMPO plants (i.e. NMP3 > MPO > NMPO). In addition, NMP3 plants produced significantly more seeds per fruit than NMPO plants (MPO plants were intermediate) in this cultivar (Table 3). In the multivariate analysis of variance, there was an overall significant treatment effect for measures of the female function, especially in 'VFNT Cherry' (MANOVA, $F = 8.83$, $P = 0.001$).

Treatment had a significant effect on mean pollen production per flower (Table 4). In 'VF36', NMP3 and MPO plants produced significantly more pollen per flower than NMPO plants. In 'VFNT Cherry', MPO plants produced significantly more pollen per flower than NMPO plants (NMP3 plants were intermediate). For total pollen production per plant, differences among the treatments were even more significant (Table 4). In both cultivars, NMP3 and MPO plants produced significantly more pollen per plant than NMPO plants. However, treatment did not have a significant effect on pollen grain size (Table 4). In the multivariate analysis of variance, there was an overall significant treatment effect for measures of the male function, especially in 'VF36' (MANOVA $F = 3.02$, $P = 0.015$).

Mycorrhizal and P responses (%) of selected vegetative and reproductive traits (Table 5) were calculated in order to compare levels of response across factors (e.g. mycorrhizal infection vs high soil P conditions, vegetative traits vs reproductive traits, female vs male, 'VF36' vs 'VFNT Cherry'). In both cultivars, P responses were higher than mycorrhizal responses for all vegetative and reproductive traits. All mycorrhizal and P responses were significant, except for the mycorrhizal response for total seed production per plant in 'VF36'. In general, reproductive responses to mycorrhizal infection and high soil P conditions were greater than vegetative responses. In 'VF36', mycorrhizal and P responses for total pollen production per plant were significantly greater than responses for leaf area and total seed production per plant (mycorrhizal response ANOVA, $F = 5.26$, $P = 0.016$; P response ANOVA, $F = 5.02$, $P = 0.021$). In 'VFNT Cherry', there was no significant difference among mycorrhizal responses (ANOVA, $F = 1.22$, $P = 0.316$). However, P responses for total seed production per plant and total pollen production per plant were significantly greater than the P response for leaf area (ANOVA, $F = 7.04$, $P = 0.005$).

Discussion

In addition to the anticipated effects on vegetative growth, this study clearly demonstrates that mycorrhizal infection and high soil P conditions also improve the female and male functions of reproduction. In many species, mycorrhizal infection enhances P uptake from the soil, thereby improving vegetative growth (Smith & Read, 1997). In this study,

similar trends in NMP3 and MPO plants and the positive correlation between leaf area and leaf P concentration across the treatments suggest that mycorrhizal effects on vegetative growth (and ultimately reproduction) were largely the result of improved P acquisition. Similarly, in separate studies with *C. pepo*, mycorrhizal infection and high soil P conditions had similar beneficial effects on the male function (Lau & Stephenson, 1994; Lau *et al.*, 1995). However, in *Cucurbita foetidissima*, low-P + AMF (arbuscular–mycorrhizal fungi) plants produced significantly more staminate flowers than low-P and high-P plants (Pendleton, 2000).

In this study, mycorrhizal infection and high soil P conditions increased both leaf area (measured before flowering) and total flower production per plant. In many species, flower production tends to increase with plant size (Jackson & Sweet, 1972; Willson *et al.*, 1979; Lloyd, 1980; Aker, 1982; Lee & Bazzaz, 1982). Similar mycorrhizal effects on total flower production have been reported for other tomato accessions (Bryla & Koide, 1990a) as well as other species (Schenck & Smith, 1982; Dodd *et al.*, 1983; Koide *et al.*, 1988; Lewis & Koide, 1990; Lu & Koide, 1994; Ganade & Brown, 1997). However, mycorrhizal effects on flower production independent of plant size have been observed in other studies. For example, Pendleton (2000) found that mycorrhizal infection increased staminate flower production in *C. foetidissima* with no effect on plant biomass.

Mycorrhizal infection and high soil P conditions improved fitness through the female function in this study. These treatments increased total fruit mass per plant by increasing total flower production per plant. Reproductive output is often highly correlated with plant size and nutrient status (Harper & White, 1974; Solbrig, 1981) and total flower production (Stephenson, 1992). Furthermore, mycorrhizal infection and high soil P conditions increased total seed production per plant by increasing fruit production (in both cultivars) and seed number per fruit (in one cultivar). In other tomato accessions, mycorrhizal infection also increased fruit and seed production and increased the percentage of flowers producing fruit (Bryla & Koide, 1990a). Similar mycorrhizal effects on fruit and seed production have been observed in several species (see Smith & Read, 1997).

In this study, mycorrhizal infection and high soil P conditions also improved fitness through the male function. These treatments increased total pollen production per plant by increasing both total flower production per plant and pollen production per flower. In natural populations, siring success is often positively correlated with flower and pollen production (Schoen & Steward, 1986; Devlin *et al.*, 1992). Moreover, pollinator attraction and subsequent pollen dissemination tend to increase with the size of the floral display (Schaffer & Schaffer, 1979; Stephenson, 1979; Willson *et al.*, 1979; Schemske, 1980a,b; Davis, 1981; Patton & Ford, 1983). Other studies have also found that mycorrhizal infection and high soil P conditions improve the male function by increasing both flower and pollen production (Lau & Stephenson, 1994; Lau *et al.*, 1995; Stephenson *et al.*, 1998; Poulton *et al.*, 2001b). For example, Pendleton (2000) found that mycorrhizal infection increased staminate flower production, and thus pollen production, in *C. foetidissima*. However, Philip *et al.* (2001) found that mycorrhizal infection increased pollen production per anther and per flower in *Lythrum salicaria* without affecting flower production.

In this study, mycorrhizal infection and high soil P conditions also decreased the number of days until first flower (related to plant size), as seen in other tomato accessions (Bryla & Koide, 1990a). In natural populations of outcrossing plants, earlier flowering could potentially improve fitness through both the female and male functions. In species with indeterminate reproduction, earlier flowering can increase total flower production by lengthening the reproductive period. In addition, plants that begin to flower earlier also begin to develop fruit earlier, possibly pre-empting resources from neighboring plants. Moreover, the pollen grains produced by the first flowers in a population have the first opportunity to fertilize ovules at a time when developing fruit are most likely to reach maturity.

Although not measured in this study, mycorrhizal infection and high soil P conditions also increase fitness through the female and male functions by improving the quality of seeds and pollen produced, as well as the quantity. During seed and pollen development, environmental conditions that affect resource availability to the sporophyte (e.g. soil fertility and leaf herbivory) can influence the quantity and quality of seeds (Roach & Wulff, 1987) and pollen produced (Stephenson *et al.*, 1994; Delph *et al.*, 1997). Several studies have found that mycorrhizal infection improves seed quality (seed size and P status), thus enhancing offspring vigor (Lewis & Koide, 1990; Koide & Lu, 1992; Lu & Koide, 1994; Shumway & Koide, 1994a,b; Heppel *et al.*, 1998). In addition, other studies have found that mycorrhizal infection and high soil P conditions improve pollen quality (*in vitro* pollen tube growth rates), thus enhancing siring success in both pollen mixture studies and experimental arrays (Lau & Stephenson, 1994; Stephenson *et al.*, 1998; Poulton *et al.*, 2001a,b).

The P responses in this study were higher than mycorrhizal responses for all vegetative and reproductive traits in Table 5. This result simply indicates that the level of P selected for NMP3 plants (based on an earlier P response study, Poulton, 2000) may have been too high. In addition, NMP3 plants received nutrient solution containing additional P throughout the growing season, while mycorrhizal hyphae were probably less effective at increasing P uptake in MPO plants as their roots became pot-bound at the end of the growing season. This reduction in the effectiveness of mycorrhizal hyphae occurred during the portion of the growing season when fruit development was at its highest, thus affecting total seed production per plant. However, the NMP3 and MPO treatments were not significantly different for most traits.

In both cultivars, mycorrhizal and P responses for reproductive traits were generally greater than responses for leaf area. However, the cultivars differed in their responses for the female and male functions. In the VF36 cultivar, mycorrhizal and P responses for total pollen production per plant were significantly greater than responses for total seed production per plant. Similarly, Pendleton (2000) found that mycorrhizal infection greatly increased staminate flower production in *C. foetidissima* with no effect on pistillate flower production. Acquisition of P (via mycorrhizal infection or other means) may be especially important for pollen grains, which showed the highest P requirement of all reproductive tissues in *Sidalcea oregana* (Ashman & Baker, 1992). For example, stored phytate in mature pollen grains is hydrolysed into phosphate and myoinositol, which are used by the pollen tube for cell wall and membrane synthesis (Jackson & Linskens, 1982; Dickenson & Lin, 1986). However, mycorrhizal and P responses of the male and female functions were not significantly different in 'VFNT Cherry'.

Despite differences in plant morphology, flowers per inflorescence, and fruit size, 'VF36' and 'VFNT Cherry' showed similar responses to treatment for many vegetative and reproductive traits measured in this study. Other tomato accessions have demonstrated significant variation in response to mycorrhizal infection (but no negative response, Bryla & Koide, 1990b). In general, cultivated varieties of tomato were more responsive to mycorrhizal infection than wild accessions, which may be better adapted to poor soil fertility (Koide *et al.*, 1988).

The evolutionary significance of mycorrhizal infection lies in its effects on plant reproduction. Selection for a trait, such as being mycorrhizal, can occur if that trait positively influences fitness. As demonstrated in the preceding study, mycorrhizal infection, similar to high soil P conditions, can increase fitness through both the female and male functions. The fact that most terrestrial angiosperm species are mycorrhizal (Law, 1988) may indicate that mycorrhizal infection does indeed increase the fitness of host plants in natural populations (Koide, 1998). Furthermore, nonmycotrophic species have developed morphological and physiological mechanisms for increasing P acquisition (e.g. proteoid roots, high root and root hair density, and acidification of the rhizosphere; Koide, 1998). Thus, the need to acquire P from the soil (by mycorrhizal infection or other means) has influenced the evolution of plant species. Understanding the impact of mycorrhizal infection on all aspects of plant reproduction, as well as vegetative growth, will become increasingly important as research on the use of mycorrhizas in agriculture, forestry, and land reclamation continues.

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